J. Sep. Sci. 2002, 25, 883–890 JOURNAL OF SEPARATION SCIENCE

Frank J. Schenck[a], **Steven J. Lehotay**[b], **Victor Vega**[a]

[a] US Food and Drug Administration, Southeastern Regional Laboratory, Atlanta, GA, USA
[b] US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA, USA

Comparison of solid-phase extraction sorbents for cleanup in pesticide residue analysis of fresh fruits and vegetables

Sample extracts of various commodities, obtained using the US Food and Drug Administration (acetone extraction) and Canadian Pest Management Regulatory Agency (acetonitrile extraction) methods for pesticides were subjected to cleanup with solid phase extraction (SPE) columns. Graphitized carbon black (GCB), octadecylsilyl (C-18), strong anion exchange (SAX), aminopropyl (—NH_2), and primary secondary amine (PSA) SPE columns were evaluated. The relative sample cleanup provided by these SPE columns was evaluated using gas chromatography with electron capture, flame photometric, and mass spectrometric detection. The —NH_2 and PSA columns were found to provide the most effective cleanup, removing the greatest number of sample matrix interferences. The GCB columns removed most of the visible plant pigment in the extracts, but did little to eliminate the fatty acid matrix interferences "seen" by the detectors. Likewise, the C-18 and SAX columns did little to eliminate matrix interferences. Using an acetone extraction followed by a PSA cleanup, both polar and nonpolar pesticides present in samples at 1.0 ng/g could be recovered.

Key Words: Solid-phase extraction; Cleanup; Pesticide residues; Analysis; Fruits; Vegetables

Received: April 17, 2001; accepted: June 5, 2002

1 Introduction

Multiresidue procedures for the determination of pesticides in fresh fruits and vegetables typically entail extraction with a water miscible organic solvent (e.g. acetonitrile or acetone), removal of the co-extracted water, and GC determination using element-selective detectors. Further cleanup of the organic solvent extract prior to GC determination is commonly performed for a variety of reasons, which include: 1) sample matrix coextractants may have a deleterious effect on the capillary GC column resulting in greater down time; 2) sample matrix coextractants may interfere with the detection of pesticides at trace levels; and 3) sample matrix coextractants may result in a sample matrix-induced enhancement effect [1, 2].

SPE columns can provide a rapid cleanup for pesticide residues in complex sample matrices. The types of SPE columns most commonly used for the cleanup of multiple types of pesticides in fresh fruits and vegetables include reverse phase sorbents such as octadecylsilyl (C-18), bonded normal phase such as aminopropyl (—NH_2) and primary-secondary amine (PSA), anion exchange such as trimethyl ammonium strong anion exchange (SAX), and adsorbents such as graphitized carbon black (GCB). Unbonded normal phase SPE columns (alumina, Florisil, and silica) are not commonly used in multi-class multiresidue methods because they strongly adsorb polar organophosphorus pesticides [3].

Many of the published methods for pesticides in fresh fruit and vegetables use combinations of two or three of the commercially available SPE columns listed above. For example, the SPE method described in the FDA Pesticide Analytical Manual uses C-18 + SAX + PSA SPE columns [4, 5]. Other combinations of SPE columns used for the cleanup of fruit and vegetable extracts include GCB + PSA [6, 7], C-18 + GCB + —NH_2 [8], and GCB + PSA + SAX [9]. This study was designed to determine the relative degree of sample cleanup obtained by using these various types of SPE columns.

Correspondence: Frank J. Schenck; FDA-SRL, 60 Eighth St. NE, Atlanta, GA 30309; USA.
E-mail: fschenck@ora.fda.gov
Fax: +1 404 253 1209

Abbreviations: SPE = solid-phase extraction; PMRA = Canadian Pest Management Regulatory Agency; ECD = electron capture detector; FDA = United States Food and Drug Administration; FPD = flame photometric detector; GCB = graphitized carbon black; MS = mass spectrometry; C-18 = octadecylsilyl; GC = gas chromatography; —NH_2 = aminopropyl; UHP = ultra high purity; SAX = strong anion exchange; PSA = primary secondary amine.

1615-9306/2002/1410–0883$17.50+.50/0

Original Paper

2 Experimental

2.1 Chromatographic instrumentation and conditions

In the case of selective detectors, two separate HP 5890 Series II GC instruments (Hewlett Packard Corp., Palo Alto, CA) were used for analysis. The first instrument was equipped as follows: ^{63}Ni ECD; packed column inlet with glass sleeve using direct injection-port mode; 30 m, 0.53 mm ID, 1.5 μm film thickness, DB-1 capillary column (J & W Scientific, Folsom, CA); UHP helium carrier gas, 20 mL/min flow rate; and 10 mL/min make-up gas of argon/methane (95+5). Injector and detector temperatures were both 250°C; column temperature was 200°C for 30 min or 200°C for 15 min and ramped at 10 K/min until the final temperature of 240°C was reached. Injection volume was 3.0 μL.

The second GC instrument was equipped as follows: FPD; 30 m, 0.53 mm ID, 1.5 μm film thickness DB-1 capillary (J & W Scientific); UHP helium carrier gas, 12 mL/min, plus 10 mL/min helium make-up gas. Injector temperature was 220°C; 225°C detector; and the oven temperature program was 130°C for 1 min and ramped at 6 K/min until the final temperature of 270°C. Injection volume was 3.0 μL.

For GC/MS, a Model 5890 Series II (Hewlett Packard) GC and a Model 5972 MS were used in the study. A 30 m, 0.25 mm ID, 0.25 μm film thickness Rtx-5MS (Restek; Bellefonte, PA) column was used with UHP helium carrier gas and 1 mL/min constant flow rate; 250°C injector; 260°C transfer line; and an oven temperature program of 75°C for 0.5 min, 25 K/min to 150°C, 6 K/min to 225°C, 25 K/min to 290°C, and held at that temperature for 6 min. Injection volume was 1.5 μL.

2.2 Materials

The pesticide standards were obtained from the US Environmental Protection Agency, Fort Meade, MD. Standard solutions of pesticides (0.20–4.0 μg/mL) were prepared in acetone. Internal standard solution of triphenyl phosphate (2.5 μg/mL) was prepared in acetone. All solvents were glass-distilled, residue grade (Burdick & Jackson, Muskegon, MI, USA). Magnesium sulfate (anhydrous powder) was ACS reagent grade (EM Science, Gibbstown, NJ, USA).

The SPE columns used were as follows: 500 mg Envi-Carb GCB, 6.0-mL size (Supelco Corp., Bellefonte, PA); 500 mg each of Bond-Elut PSA, $-NH_2$, and SAX, 10-mL LRC (laboratory robot compatible) size, and 1000 mg Mega Bond Elut C-18, 6-mL size (Varian Sample Preparation Products; Harbor City, CA, USA).

2.3 Sample extraction

Apple, asparagus, cabbage, collard green, grape, green pepper, plum, and squash samples were extracted by the FDA method, as follows. Sample (50 g) was weighed into a blender cup and extracted by the method of Luke et al. as described elsewhere [3, 4]. Briefly, the sample was blended with 100 mL acetone and filtered through coarse filter paper. A 40-mL portion of the aqueous acetone extract was subjected to partitioning with 100 mL 1/1 methylene chloride/petroleum ether. NaCl was added to the aqueous layer after the first partitioning, and the extract was partitioned two times with 50 mL methylene chloride. The sample was concentrated on a steam bath in the presence of petroleum ether, and then acetone, to remove all traces of methylene chloride. The extract was adjusted to 1 mL final volume with acetone.

Apple, green pepper, plum, and collard green samples were extracted using a procedure based on the Canadian PMRA method [8]. Sample (50 g) was weighed into a blender cup and blended with 100 mL of acetonitrile. The sample was filtered through coarse filter paper, and the filtrate was transferred into a 1-L separatory funnel. NaCl (5 g) was added, the funnel was shaken for one min, and the phases were allowed to separate for 15 min. The lower aqueous phase and any emulsion were discarded. Anhydrous magnesium sulfate (4 g) was added to the separatory funnel, and the funnel was shaken for 30 s. The dried extract was filtered through coarse filter paper, and 50 mL of the extract was evaporated to <1 mL under nitrogen at 55°C in a TurboVap Evaporator (Zymark Corp., Hopkinton, MA). The extract was adjusted to 1 mL final volume with acetonitrile.

2.4 SPE cleanup

Elution solvents for the SPE columns were as follows: acetonitrile for C-18; 3/1 (*v/v*) acetone/toluene for GCB; and acetone for PSA, SAX, and $-NH_2$. When the C-18 +GCB+SAX cleanup was used, the extract was first eluted through the C-18 with acetonitrile. The eluate was evaporated almost to dryness, dissolved in acetone/toluene, and eluted through tandem GCB+SAX columns with acetone/toluene.

Magnesium sulfate (ca. 2 cm layer) was always added to top of the SPE columns, and the individual columns were preconditioned with elution solvents. Vacuum was adjusted such that the flow rate was 1–2 drops per second, and the eluates were collected in 15-mL conical centrifuge tubes. Once all the extract reached the sorbent bed, the columns were eluted with two 5-mL portions of elution solvent. The eluates were evaporated to <1.0 mL with the aid of a stream of nitrogen at 55°C. If the eluates contained solvents other than acetone, two 10-mL por-

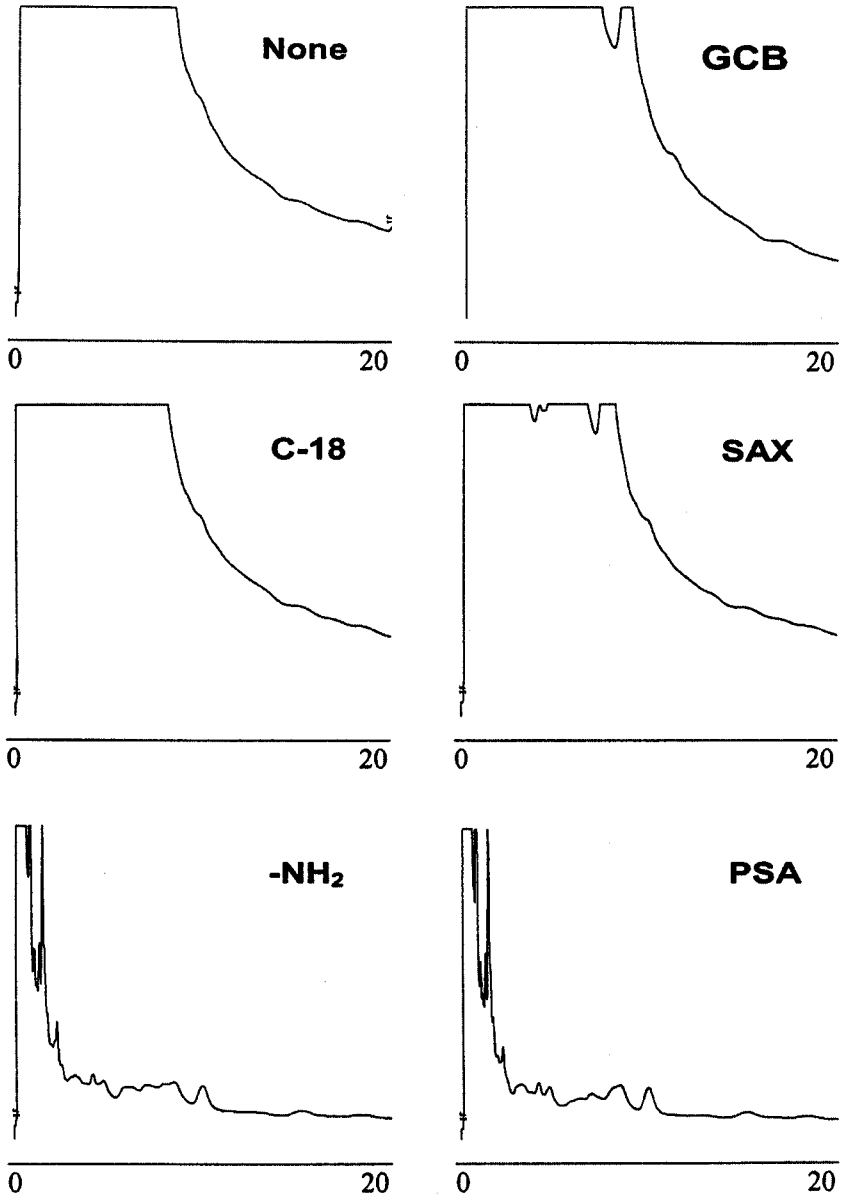

Figure 1. GC/ECD chromatograms before and after SPE cleanup of a blank asparagus extracted by the FDA method (acetone extraction).

tions of acetone were added and the eluates were evaporated under nitrogen to <1.0 mL after each addition to make a solvent exchange to acetone.

The final volumes of the extracts were adjusted with acetone to 10 mL if the extracts were to be analyzed on GCs equipped with an ECD or MS. Final extracts were equivalent to 1.4 g sample equivalent/mL of final extract. If the extracts were to be analyzed by GC/FPD, the final volume of the extracts was adjusted to 0.4 mL to give a final equivalent concentration of 35 g sample equivalent/mL of final extract.

The following study was performed to determine the recovery of pesticides through SPE cleanup. Six blank grape sample extracts were obtained using the FDA method. Four of these extracts were then spiked with 70 μL of mixed standard. The four spiked and two blank extracts were then taken through an SPE cleanup. Matrix standards were then prepared by spiking the two blank extracts with 70 μL of mixed standard. Internal standard

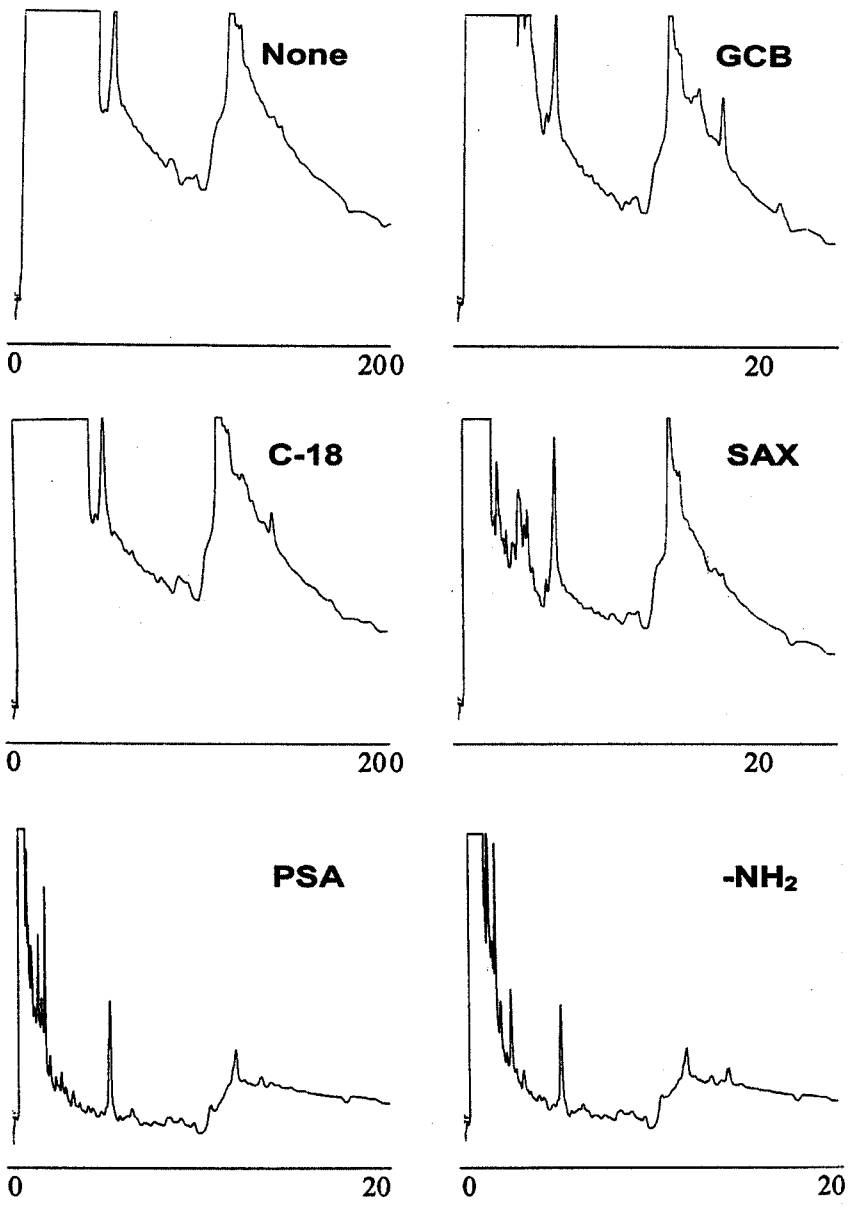

Figure 2. GC/ECD chromatogram before and after SPE cleanup of a blank green pepper extracted by the Canadian PMRA method (acetonitrile extraction).

(50 μL) was added to both the matrix standards and spikes, the final volumes were adjusted to 0.4 mL, and they were analyzed by GC/FPD.

3 Results and discussion

3.1 SPE sample cleanup as determined by GC/ECD and GC/MS

Acetonitrile or acetone extracts of fresh fruit and vegetable samples were eluted through reverse phase (C-18), bonded normal phase (—NH_2 and PSA), strong anion exchange (SAX), and carbon (GCB) SPE columns. The SPE sorbents thus served as chemical filters, retaining the matrix coextractants while allowing the pesticides to elute. Previous studies demonstrated that the solvents used for elution from these SPE columns achieved high pesticide recoveries [6, 8], which was why they were chosen for use in this study.

The SPE columns were topped with magnesium sulfate to remove any residual water that may have been present in

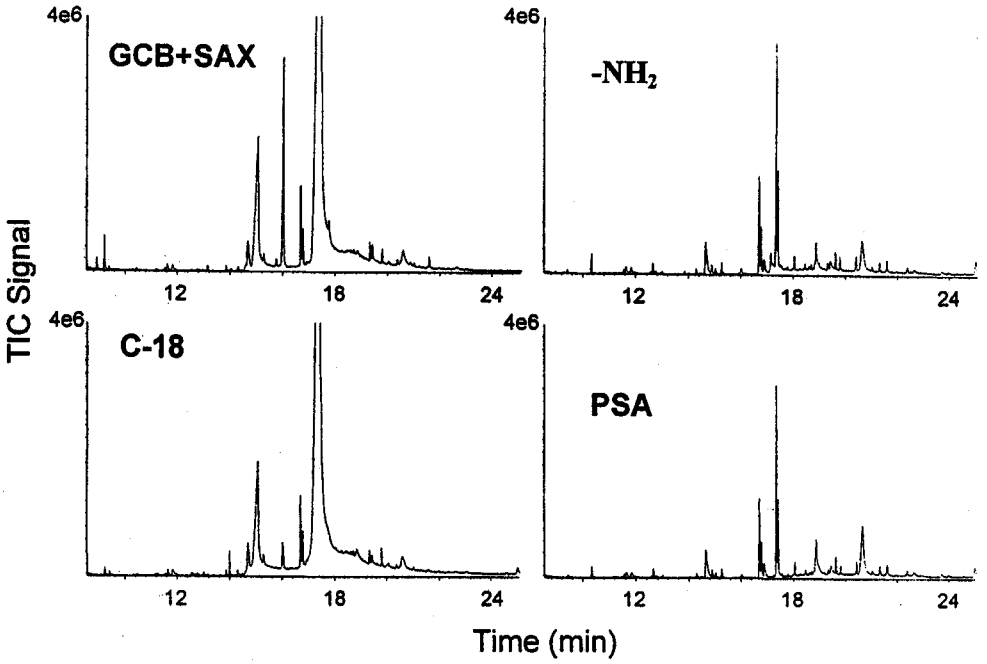

Figure 3. GC/MS total ion chromatograms after SPE cleanup of a blank green pepper extracted by the FDA method (acetone extraction).

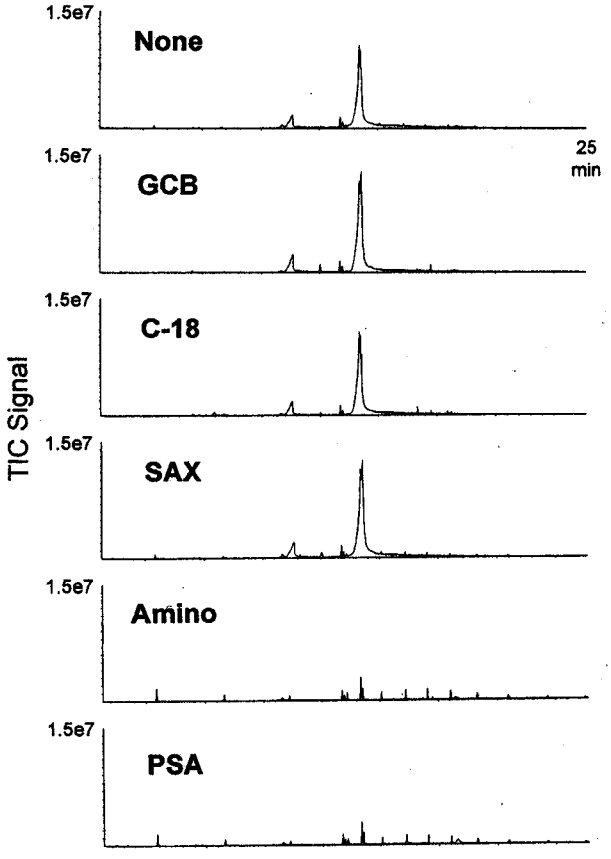

Figure 4. GC/MS total ion chromatograms before and after SPE cleanup of a blank green pepper extracted by the Canadian PMRA method (acetonitrile extraction).

the sample extracts. It had been reported [6] and we confirmed that magnesium sulfate is effective in improving SPE cleanup of acetone extracts of fruits and vegetables. Sodium sulfate, which is most commonly used for this purpose, was not used since it has been shown to be relatively ineffective in removing water from acetone/water or acetonitrile/water mixtures [10, 11].

The relative cleanup achieved with the various SPE columns was evaluated in GC/ECD, FPD, and MS analysis. **Figure 1**, **Figure 2**, **Figure 3**, and **Figure 4** show that the bonded normal phase SPE columns (—NH_2 and PSA) were the most effective in removing the matrix coextractants from both FDA and PMRA sample extracts. From the standpoint of visual appearance only, the GCB column seemed to have the greatest effect. Even though all the extracts that had been subjected to a GCB cleanup were transparent and almost colorless, Figure 1, Figure 2, Figure 3, and Figure 4 show that the GCB had very little effect on removing the coextractants detected by these two detection systems. Interestingly, an earlier study [2] had shown that the PSA and —NH_2 SPE columns were also the most effective in reducing the matrix enhancement effect in the GC analysis of pesticides, while the GCB column had little effect on reducing matrix-induced enhancement. Since matrix-induced enhancement is an "injector effect", this would mean that the pigments being removed by the GCB do not have strong interactions in the injector. Likewise, the C-18 and SAX also removed relatively little of the co-eluting matrix coextractants present in any of the samples tested.

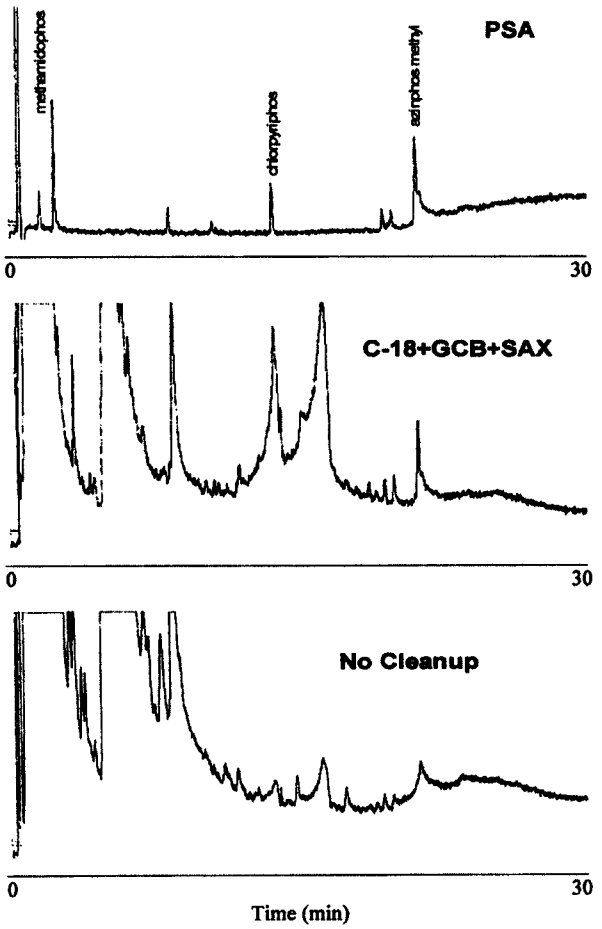

Figure 5. GC/FPD chromatograms before and after SPE cleanup, of asparagus spiked at 1.0 ng/g with the pesticides azinphos methyl, chlorpyriphos, and methamidophos, extracted by the FDA method.

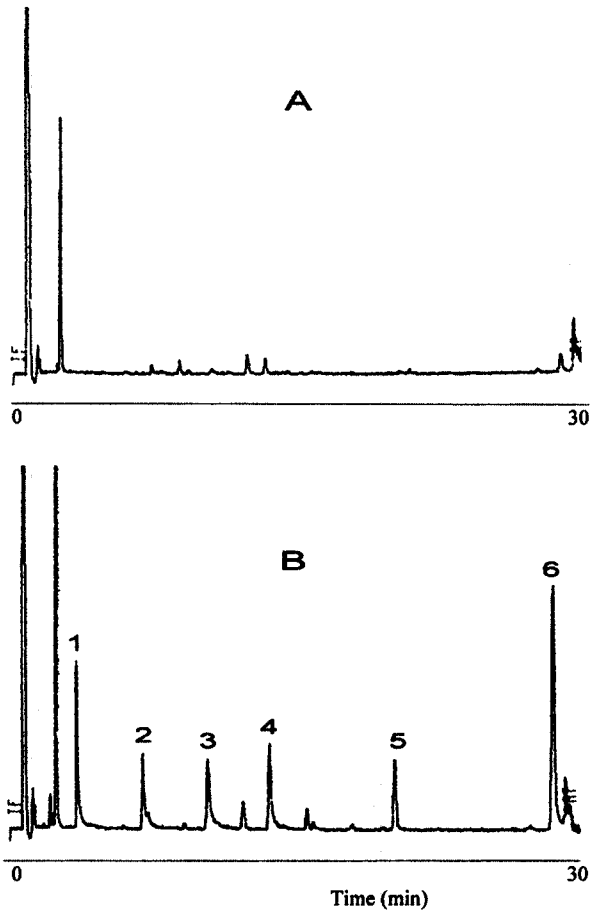

Figure 6. GC/FPD chromatograms of (A) a control grape sample and (B) a control grape sample spiked with pesticides at 1.0 ng/g, extracted by the FDA method and subjected to a PSA SPE cleanup. Peak identities are (1) methamidophos, (2) acephate, (3) omethoate, (4) dimethoate, (5) chlorpyriphos, and (6) tri phenyl phosphate (internal standard).

Mass spectrometry also revealed that the main constituents removed by the PSA and $-NH_2$ columns were fatty acids, such as hexadecanoic and octadecanoic acids. None of the other columns removed these large peaks in the chromatograms, which were especially prevalent in the green vegetables.

3.2 SPE sample cleanup as determined by GC/FPD

While the ECD and MS systems gave an indication of the gross cleanup of matrix coextractants, which would

Table 1. Recoveries through SPE cleanup of organophosphorus pesticide residues from fortified grape extracts obtained using the FDA method.

	PSA cleanup		C-18+GCB+SAX cleanup	
	Fortification (ng/g)	% Recovery[a)]	Fortification (ng/g)	% Recovery[a)]
Acephate	1.0	76.9 (6.1)	30	54.0 (14.3)
Azinphos methyl	5.0	95.5 (3.6)	30	73.1 (10.3)
Chlorpyrifos	1.0	94.6 (4.6)	30	76.5 (12.9)
Dimethoate	1.0	94.1 (4.0)	30	86.5 (9.0)
Methamidophos	1.0	70.8 (6.4)	30	89.1 (6.7)
Omethoate	1.0	84.4 (8.2)	30	95.8 (9.6)

a) $n = 4$, values in parentheses are coefficient of variation.

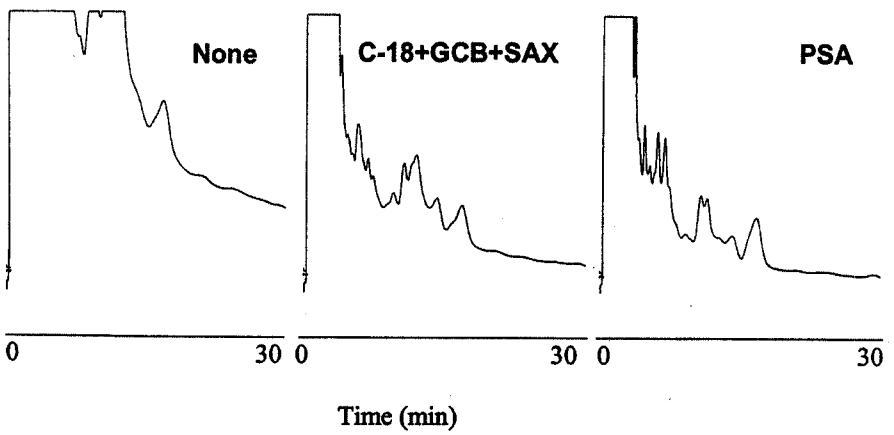

Figure 7. GC/ECD chromatograms before and after SPE cleanup of a blank cabbage extracted by the FDA method (acetone extraction).

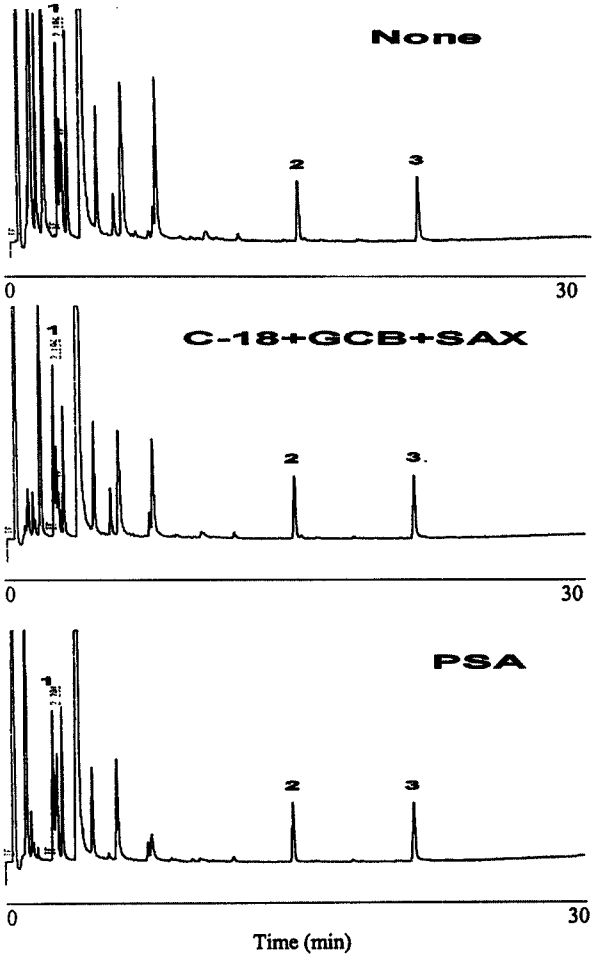

Figure 8. GC/FPD chromatograms before and after SPE cleanup, of cabbage spiked at 10.0 ng/g and extracted by the FDA method. Peak identities are (1) methamidophos, (2) chlorpyriphos, (3) triphenyl phosphate.

reduce the need for system maintenance, the question remains what effect if any do these coextractants have on the analysis of the samples for pesticides residues. We analyzed a series of samples that had been spiked with organophosphorus pesticides in the low ng/g range with the FPD. We found again that using —NH_2 and PSA SPE columns resulted in a better cleanup than found with the other SPE columns. **Figure 5** and **Figure 6** shows how the PSA SPE cleanup was very effective in removing sample matrix coextractants, making it possible to detect pesticide residues in some foods at 1.0 ng/g.

Certain foods like cabbage, which contain organosulfur compounds, have always been problematic with the FPD, since it will detect organosulfur as well as organophosphorus compounds. None of the SPE columns evaluated in this study would efficiently remove the organosulfurs from cabbage extracts. **Figure 7** and **Figure 8** show that even though the SPE columns removed many of the matrix coextractants from a cabbage sample, sufficient organosulfur compounds remained to interfere with the detection of early eluting organophosphorus pesticides with the FPD.

Of course, the SPE would not be suitable if the both the pesticides and matrix coextractants were retained on the SPE column. **Table 1** shows that organophosphorus pesticides ranging from the very polar acephate and methamidophos to relatively nonpolar chlorpyriphos could be easily recovered through both the PSA SPE column as well as a combination of C-18 + GCB + SAX using the conditions given.

4 Conclusions

The main conclusion of this study is that the use of a single bonded normal phase SPE column, —NH_2 or PSA, can provide an excellent cleanup of fresh fruit and vegetable extracts for multiresidue pesticide analysis. The GCB sorbent also removes pigments, but does not remove noticeable chromatographic interferants. Thus, —NH_2 or PSA

alone can achieve acceptable cleanup of extracts, and the use of common additional SPE columns provides little benefit and essentially serves to increase the cost of analysis and solvent consumption.

References

[1] D.R. Erney, A.M. Gillespie, D.M. Gilvydis, C.F. Poole, *J. Chromatogr.* **1993**, *638*, 57–63.

[2] F.J. Schenck, S. Lehotay, *J. Chromatogr. A* **2000**, *868*, 51–61.

[3] M. Luke, J.E. Froberg, H.T. Masumoto, *J. Assoc. Off. Anal. Chem.* **1975**, *58*, 1020–1026.

[4] PAM, *Pesticide Analytical Manual, Volume I, 3rd edition, 1994, updated October, 1999*, U.S. Food and Drug Administration, Washington, DC. Section 302 http://vm.cfsan.fda.gov/~frf/pami3.html.

[5] T. Cairns, M.A. Luke, K.S. Chiu, D., Navarro, E.G. Siegmund, *Rapid Comm. Mass Spectrom.* **1993**, *7*, 1070–1076.

[6] L.V. Podhorniak, J.F. Negron, F.D. Griffith, Jr., *J. AOAC Int.* **2000**, *84*, 873–890.

[7] F.J. Schenck, V. Howard-King, *Bull. Environ. Contam.-Toxicol.* **1999**, *63*, 277–281.

[8] J. Fillion, F. Sauvé, J. Selwyn, *J. AOAC Int.* **2000**, *83*, 698–713.

[9] R. Sheridan, J. Meola, *J. AOAC Int.* **1999**, *82*, 982–990.

[10] S.J. Lehotay, A.R. Lightfield, J.A. Harman-Fetcho, D.J. Donoghue, *J. Agric. Food Chem.* **2001**, *49*, 4589–4596.

[11] F.J. Schenck, P.S. Callery, P.M. Gannett, J.R. Daft, S.J. Lehotay, *J. AOAC Int.* (in press).

[JSS 1250]